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TITLE: Evaluation of Listeria Monocytogenes Based Vaccines for HER-2/neu in Mouse Transgenic Models of Breast Cancer

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14. ABSTRACT The potential benefits of antiangiogenic therapy have been dramatically shown in mouse tumor models but have been less striking in human clinical trials. A possible explanation for this disparity in treatment outcomes is that the vasculature of human tumors may be more resistant to antiangiogenic therapies. This may be due, at least in part, to extensive pericyte coverage of vessels in many common human cancers, such as breast cancers, compared to a relative deficiency of pericytes surrounding vessels in commonly studied mouse tumors. We have identified two autochthonous mouse mammary tumor models, MMTV-infected and MMTV-neu mice, with high pericyte coverage of tumor vessels that may better recapitulate human breast cancer. The endothelial-specific receptor tyrosine kinase, Tie2, regulates microvessel pericyte coverage and activates endothelial cell (EC) signal transduction pathways that promote their survival (e.g. the PI3 kinase-AKT signaling pathway). Our previous studies using an inducible decoy receptor of Tie2 (Tie2Ex) to inhibit Tie2 activation in K1735 murine melanoma tumors showed a decrease in activated AKT expression in ECs and increased EC apoptosis. Tie2Ex expressing tumors also had decreased pericyte coverage, suggesting Tie2 inhibition in tumors can destabilize vessels. We have generated transgenic MMTV-infected and MMTV-neu mice that express Tie2Ex to inhibit Tie2 activation in mammary tissues. The presence of Tie2Ex does not appear to affect EC pAKT or pERK expression downstream of Tie2 signaling in mammary glands. We are currently awaiting tumors to develop in these transgenic mice to study the effect of Tie2 inhibition in mammary tumors.					
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Proposal Title: Overcoming Obstacles to Anti-Angiogenic Therapy in Breast Cancer

Introduction

Antiangiogenic therapy of cancers involves inhibiting tumor blood vessel development to deprive tumors of vital oxygen and nutrients. The potential benefits of antiangiogenic strategies have been dramatically shown in mouse tumor models. Results in human clinical trials, however, have been less striking. Recent trials have shown survival benefits, but tumor regression, which is often reported in murine tumors, is rarely seen in treated human cancers. A potential explanation for this disparity in treatment outcomes is that the vasculature of human tumors may be more resistant to antiangiogenic therapies. This may be due, at least in part, to extensive pericyte coverage of vessels in many common human cancers, such as breast cancers, compared to a relative deficiency of pericytes surrounding vessels in commonly studied mouse tumors. Pericytes are periendothelial mesenchymal cells that surround capillaries. Their presence is associated with microvessel maturity and stability and may confer resistance to certain antiangiogenic agents. Mouse mammary tumor virus (MMTV)-induced mammary carcinomas arising in C3H/HeN mice and transgenic mice expressing wildtype HER2/neu under the MMTV promoter (MMTV-neu) may more faithfully model human breast cancer vasculature inasmuch as vessels in these tumors have extensive pericyte coverage like in human breast cancers. Interestingly, we found the MMTV-induced mammary carcinomas were resistant to rIL-12 antiangiogenic therapy, which was effective against every other mouse tumor model tested (Lee JC et al., *Canc. Res.* 62: 747-755. 2002).

The endothelial-specific receptor tyrosine kinase, Tie2, regulates microvessel pericyte coverage, as well as activating endothelial cell (EC) signal transduction pathways that promote their survival (e.g. the PI3 kinase-AKT signaling pathway). Our previous studies using an inducible decoy receptor of Tie2 (Tie2Ex) to inhibit Tie2 signaling in K1735 murine melanoma tumors showed a decrease in activated AKT expression in ECs and an increase in EC apoptosis. Tie2Ex expressing tumors also had decreased pericyte coverage, suggesting Tie2 inhibition can destabilize vessels. Studies proposed in this training grant examine whether inhibiting Tie2 activation diminishes pericyte coverage and apoptosis-resistance in mouse mammary tumors and enhance their susceptibility to antiangiogenic therapy. Our strategy was to develop an inducible system for inhibiting Tie2 activation by expressing Tie2Ex in MMTV-induced and MMTV-neu transgenic mice.

Body of Report

Task 1. Generate mammary specific inducible Tie2Ex mice

a. Cross MMTV-rtTA mice with TRE-Tie2Ex mice (FVB and C3H strain).

The Transgenic Mouse Facility at Penn generated C3H/HeN TRE-Tie2Ex and FVB TRE-Tie2Ex transgenic mice using a TRE-Tie2Ex plasmid. Mouse lines that stably transmitted the transgene were identified and crossed with MMTV-rtTA transgenic mice of the FVB strain (provided by Lewis Chodosh, University of Pennsylvania), which express rtTA in mammary and certain other tissues. Double transgenic (MMTV-rtTA⁺, TRE-Tie2Ex⁺) F1 female progeny showed high-level doxycycline-inducible Tie2Ex expression in mammary glands and certain other organs (e.g. salivary glands) (Figure 1).

b. Characterize effect of Tie2Ex induction on normal vessels in normal mammary glands.

MMTV-rtTA⁺, TRE-Tie2Ex⁺ mice littermates were designated to receive doxycycline (dox) at 2mg/ml or no dox in the drinking water for one week to induce or not induce Tie2Ex respectively. Mice were then euthanized and mammary glands were removed and processed for histological analysis. Because Tie2 can activate EC signaling transduction pathways, such as the Raf-MEK-ERK and PI3K-AKT pathway, we stained mammary tissue sections from Tie2Ex induced and non-induced mammary glands for phospho-ERK (pERK) or phospho-AKT (pAKT) followed by CD34 to identify EC pERK or pAKT expression. These analyses revealed no significant changes in the EC pERK expression or EC pAKT expression in the presence or absence of Tie2Ex (Figure 2). We are currently analyzing mammary glands from mice receiving dox for 1 month to see whether length of induction will alter EC signaling.

c. Cross double transgenic MMTV-rtTA TRE-Tie2Ex mice with MMTV-neu mice and wait for tumors to develop (FVB strain).

MMTV-rtTA TRE-Tie2Ex FVB mice were crossed with MMTV-neu mice to generate mammary tumors with the inducible Tie2Ex gene. Triple transgenic mice at 6 weeks of age were force bred until 5-6 months of age to enhance MMTV mammary tumorigenicity. Some of the mice have recently developed tumors and tumor fragments were transplanted into SCID recipient mice to passage and to expand the tumors for study. To test the ability of dox to induce Tie2Ex in these mammary tumors, primary tumor fragments were cultured *ex vivo* in the presence of dox in the media. Tie2Ex was detected in the supernatant only in the presence of dox, indicating that these tumors contain the inducible transgene and can be used to study the effect of Tie2 inhibition in mammary tumors.

d. Cross MMTV-rtTA transgenic mice with MMTV infected TRE-Tie2Ex transgenic mice and wait for tumors to develop (C3H strain).

To generate mice with MMTV-induced tumors that carry the MMTV-rtTA and TRE-Tie2Ex transgenes, we crossed FVB MMTV-rtTA male transgenic mice with C3H/HeN TRE-Tie2Ex female transgenic mice that were infected with mouse mammary tumor virus. As both transgenic lines are heterozygous and only female mice develop MMTV-induced tumors, only 1 in 8 pups were double transgenic females. These were infected with MMTV virus transmitted via mother's milk transmission and beginning at 6 weeks of age were force bred until 5-6 months of age to enhance MMTV mammary tumorigenicity. The oldest of the retired breeder, double transgenic mice are now 9-10 months old, but none have yet developed tumors.

Task 2. Evaluate effect of Tie2 inhibition on mammary tumors.

MMTV-rtTA⁺ TRE-Tie2Ex⁺ neu⁺ (FVB) mice recently developed tumors which we transplanted into SCID recipient mice to passage and to expand the tumors for study. These primary tumor fragments were cultured *ex vivo* in the presence of dox and showed induction of Tie2Ex. We are currently awaiting the transplanted tumors to develop.

MMTV-rtTA⁺ TRE-Tie2Ex⁺ (C3H) mice infected with MMTV virus that were previously force bred to enhance MMTV mammary tumorigenicity are 9-10 months old have not yet developed tumors. Once tumors develop in these mice, fragments of these tumors will be transplanted into SCID recipient mice to passage and expand these tumors for study. A potential explanation for the delay in mammary tumor formation is that these mice are C3H/HeN x FVB F1 and may develop tumors with different kinetics than C3H/HeN mice.

Key Research Accomplishments

We have created double TRE-Tie2Ex x MMTV-rtTA transgenic mice (FVB and C3H strain) that show robust Dox-induced Tie2Ex expression in their mammary glands.

Established Tie2Ex induction in normal mammary gland does not affect EC pAKT or pERK expression after one week of induction.

Generated mammary tumors from MMTV-rtTA⁺ TRE-Tie2Ex⁺ neu⁺ (FVB) mice and transplanted into recipient mice.

Reportable Outcomes

A manuscript is being prepared based on work done on inducible Tie2Ex in K1735 cells and tumors and will soon be submitted.

Jeff Tsai will be defending his doctoral thesis in December 2007.

Conclusions

Our previous studies suggest inhibition of Tie2 activation by Tie2Ex expression inhibits tumor endothelial cell signaling through the PI3K-AKT pathway. We have developed transgenic mice that inducibly express Tie2Ex in mammary glands and see no change in pAKT or pERK expression after one week of induction. We are currently awaiting tumors to develop from MMTV-induced and MMTV-neu transgenic mice with the inducible Tie2Ex transgene to study the effect of Tie2 inhibition in mouse mammary tumors

References and Appendices

Presentation P19-9 at Era of Hope meeting, Philadelphia, PA (6/8/05 - 6/11/05).

Supporting data

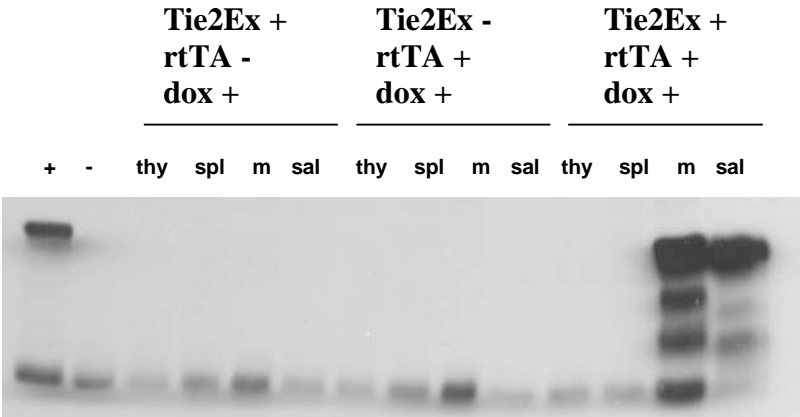


Figure 1- Expression of Tie2Ex in mammary and salivary glands. Doxycycline (2mg/ml) was added to the drinking water of transgenic mice for 48hrs. Thymus (thy), Spleen (spl), mammary gland (m), and salivary gland (sal) were excised and homogenized in lysis buffer. Tie2Ex protein expression was detected using an anti-Tie2 antibody on a western blot from tissue lysate.

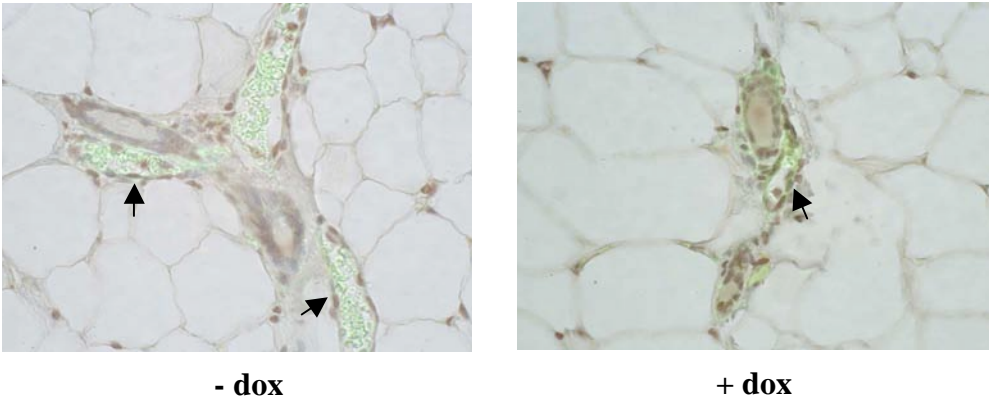


Figure 2- EC pAKT expression in mammary tissues. Mammary glands were excised from mice receiving no dox (left) or dox (right) for one week. Tissue sections from paraffin-embedded mammary glands were stained for pAKT (brown) using immunohistochemistry and CD34 (green) using immunofluorescence. Arrows indicate EC nuclei positive for pAKT expression. 40X